

Serial No.: 08/338,730  
Filed: 14 November 1994

**Provisional Double Patenting Rejection**

The provisional double patenting rejection is noted and will be addressed at the appropriate time (i.e. upon an indication of allowable subject matter in this or one of the co-pending applications).

**Rejection under 35 U.S.C. § 112, 2nd ¶:**

The Examiner rejected claims 1-10 under § 112, 2nd ¶, finding the phrase "and/or" vague and unclear because "either one or two different factors may be required...". The phrase was not intended to mean that the use of one of the factors is optional, but rather that the method of regulation as outlined in amended claim 1 will a) effect the proliferation of multipotent neural stem cells or b) effect the proliferation of the precursor cell progeny of the multipotent neural stem cells or c) effect the proliferation of both multipotent neural stem cells and the precursor cell progeny of the multipotent neural stem cells. This is described in detail in the specification on page 13, lines 1-21. Claim 1 has been amended to clarify that the culture medium contains both a proliferative factor and additionally contains at least one regulatory factor. Claim 1 now recites that the regulatory factor "regulates multipotent neural stem cell proliferation and/or regulates precursor cell proliferation." The term "precursor cells" refers to the progeny of a multipotent neural stem cell, and includes daughter multipotent neural stem cells and daughter progenitor cells. Support for this term is in the specification on page 1, lines 20-21. Accordingly, it is believed that the rejection is overcome.

Serial No.: 08/338,730  
Filed: 14 November 1994

**Rejections Under 35 U.S.C. § 103**

Applicants acknowledge the duty to disclose the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made. The invention defined by the claims of the present application was commonly owned when made.

Claims 1-10 were rejected under § 103 as being unpatentable over Boss taken with Anchan, Lin, Ferrari and Morshead.

***Claim 1***

The Examiner stated that "Boss discloses that his cell population contains neural stem cells since (column 6) "... cells migrating from these (3-D) structures and form typical two dimensional monolayers in which differentiating neurons and glial cells can be observed." However, the fact that glial cells and neurons were observed in the cultures of Boss does not mean that multipotent neural stem cells were induced to proliferate as required by Claim 1.

Boss discloses methods of culturing neuronal progenitor cells obtained from ventral mesencephalon tissue. Approximately 3000 cells are initially plated into each tissue culture well (see col. 9, line 10). It would be expected that each well would initially contain many types of cells, including committed progenitor cells for both neurons and glial cells. Thus, Boss's observations regarding the presence of neurons and glia does not mean that multipotent neural stem cells proliferated, as these results can be explained by the differentiation of committed neuronal and glial progenitor cells. The

**Serial No.:** 08/338,730  
**Filed:** 14 November 1994

differences between neural stem cells and progenitor cells are set forth in Applicants' specification on page 9, lines 15-29.

While it is possible that neural stem cells were present in Boss's primary culture, claim 1 requires more than the mere presence of a stem cell. The induction of proliferation of the neural stem cells present is also required. Once a stem cell is placed in a culture medium *in vitro*, several fates of the cell are possible depending on the culture conditions. The stem cell may: 1) die, 2) proliferate to produce progeny cells, or 3) survive the culture conditions, but not proliferate (i.e. remain relatively quiescent). There is no indication from the Boss reference that stem cells were induced to proliferate using the culture conditions described. In fact, Boss specifically selected for neuronal progenitor cells by plating cells in an initial selection medium. After this selection step, the selected neuronal progenitor cells were proliferated in a second culture medium (see last sentence of Boss abstract).

That the selected cells which were proliferated using the Boss method were neuronal progenitor cells and not multipotent neural stem cells is further illustrated by the limitations provided in claim 1 of the Boss patent in which it is stated "... said progenitor cells in said culture being capable of seven to eight rounds of cell division after dissociation...". As outlined in Applicants' specification (see bottom of page 1), progenitor cells are capable of limited proliferation, whereas stem cells are capable of self-maintenance, meaning that they are capable of undergoing essentially limitless rounds of cell division.

Serial No.: 08/338,730  
Filed: 14 November 1994

The Examiner also stated that "Anchan discloses a method for the in vitro proliferation of neural stem cells..." and cited page 923, column 2, of Anchan under the heading "Results". However, the referenced portion does not concern neural stem cell proliferation. It describes primary cultures of embryonic and neonatal retinal cells and concludes that immunohistochemistry indicated that "many of the cells that are actively dividing in the cultures are neuronal progenitors." As noted above with regards to the Boss reference, a neuronal progenitor cell is not a multipotent neural stem cell.

Thus, neither one of the references relied upon by the Examiner for the teaching that multipotent neural stem cells can be cultured *in vitro* teach or suggest that this is possible. Instead, both of these references teach the *in vitro* proliferation of neuronal progenitor cells. While it is believed that the rejection of claims 1-10 should be withdrawn on this basis alone, Applicants further discuss below additional differences that exist between the claimed invention and the cited prior art.

Claim 1 requires that the proliferated multipotent neural stem cell is capable of producing progeny are capable of differentiating into neurons, astrocytes and oligodendrocytes. While Boss states that glial cells were observed in her cultures, there is no indication that 1) both astrocytes and oligodendrocytes were observed and 2) that the glial cells were the progeny of a multipotent neural stem cell induced to proliferate *in vitro* by at least one proliferative factor. Anchan cultured embryonic cells that terminally differentiated into neurons and he cultured cells from neonate tissue that terminally

Serial No.: 08/338,730  
Filed: 14 November 1994

differentiated into neurons and Müller cells. The cells cultured by Anchan did not produce progeny which differentiated into neurons, astrocytes and oligodendrocytes. Therefore, this is an additional feature of claim 1 that is not taught or suggested by the references cited by the Examiner.

***Claim 3***

The Ferrari reference is cited for disclosing that "bFGF promotes survival and development of neurons in culture." Applicants speculate that this reference is being applied against claim 3 which recites that the proliferative factor is bFGF. However a reference that teaches that a growth factor that promotes the survival of neurons, which are terminally differentiated cells, does not teach or suggest that the same growth factor will induce proliferation of stem cells, which are undifferentiated cells. As noted above, given a particular set of culture conditions, a particular cell may: 1) die, 2) proliferate to produce progeny cells, or 3) survive the culture conditions, but not proliferate. Thus, with respect to neurons, the Ferrari reference teaches that bFGF has the third effect. However, it should be emphasized that mere survival is not the same as proliferation.

***Claim 5***

It is not understood how the first sentence of the middle paragraph on page 5 of the office action relates to the claims of the present application. The claims are not concerned with inducing dopamine expression or transplantation of cells.

Serial No.: 08/338,730  
Filed: 14 November 1994

Claim 5 requires that the regulatory factor is heparin sulfate and depends on claim 3 which requires that the proliferative factor is bFGF. Accordingly, claim 5 requires, *inter alia*, that a multipotent neural stem cell is proliferated in a culture medium containing bFGF and heparin sulfate. The rejection of this claim cannot be maintained. The Examiner stated that "the use of hepar(a)n sulfate is obvious over the use of other growth factors since the ***specification discloses*** that hepar(a)n sulfate promotes binding of bFGF, for example, to its cell surface receptor." (emphasis added). The Examiner cannot use the Applicants' own disclosure to make an obviousness rejection. Rather, a proper obviousness analysis requires that:

the scope and content of ***the prior art*** are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined.

*Graham v. John Deere Co.*, 148 USPQ 459, 467 (S.Ct. 1966).  
Accordingly, the Examiner's rejection of this claim is improper and should be withdrawn.

***Claim 6***

Claim 6 requires that the regulatory factor is EGF and is dependent upon claim 3 which requires that the proliferative factor is bFGF. This particular combination results in the proliferation of more multipotent neural stem cells than with EGF alone (as indicated by a larger number of neurosphere formation) and induces a faster rate of proliferation than with bFGF alone (as indicated by

**Serial No.:** 08/338,730  
**Filed:** 14 November 1994

the generation of larger spheres) (see page 18, lines 8-19; and Table I on page 19).

The Examiner stated that "the modification of the method of Boss by adding different growth factors as suggested by Anchan, Lin, Ferrari and Morshead... was well within the ordinary skill in the art at the time the claimed invention was made." However, there are many different growth factors. The Examiner has not provided any reasoning why the particular combination of EGF with bFGF, would have been selected by one of ordinary skill in the art. Even if it could be said that it would be obvious to try various combinations of growth factors to determine if various combinations result in additive or synergistic effects, "obvious to try" is not the appropriate test. See *In re Fine*, 5 USPQ2d 1596, 1599 (CAFC 1988). The Examiner has not also provided any reasoning why one of ordinary skill in the art would have had a reasonable expectation that bFGF would modify the proliferative effects of EGF. Accordingly, the rejection of claim 6 cannot be maintained.

#### ***Claim 8***

With regards to claim 8, the Examiner cited Morshead for disclosing that "the adult brain contains cells capable of dividing in a stem cell mode." The Examiner stated that "it would have been obvious... to modify the method of Boss by using cells derived from adult tissue." There are several reasons why one of ordinary skill in the art would not have combined the teachings of Boss with Morshead. First, Boss is concerned with culturing neuronal progenitor cells of the mesencephalon, a brain region that is rich in dopaminergic cells in the adult mammal. Boss teaches that the

Serial No.: 08/338,730  
Filed: 14 November 1994

neuronal progenitor cells are obtained "at the *appropriate stage* of embryonic development." (col. 4, lines 1-4; emphasis added). The appropriate stage is at "the early stages of development, prior to neurite formation." (col. 5, lines 24-25). This implies adult neural tissue would not be an appropriate tissue to use in the culture method of Boss. Another reason why one would not have combine the two references in the manner suggested by the Examiner is that Morshead studied dividing cells of the subependymal layer of the brain. This is part of the striatum, which, in the adult mammal, does not normally contain dopaminergic cells. Boss was concerned with the proliferation of neuronal progenitor cells that differentiate into dopaminergic cells. Accordingly, one of ordinary skill in the art would not have modified the method of Boss by using the adult tissue studied by Morshead, because it would not be expected that the modification would result in the proliferation of dopaminergic cells.

Even if the two references were combined, it would not result in the claimed invention. As stated above, Boss does not teach the proliferation of multipotent neural stem cells *in vitro* that are capable of producing progeny that can differentiate into neurons, astrocytes, and oligodendrocytes. This missing feature (among others) cannot be found in Morshead either. Morshead describes experiments in which mitotically active cells present in the subependymal region of the adult mammalian forebrain were labeled with BrdU. The study indicated that one of the progeny from each cell division dies. Morshead did not identify whether these dividing, undifferentiated cells could be induced to differentiate. The reference would not have suggested to one of



Serial No.: 08/338,730  
Filed: 14 November 1994

ordinary skill in the art that multipotent neural stem cells capable of producing progeny that can differentiate into neurons, astrocytes and oligodendrocytes are present in adult mammalian neural tissue, and further that such stem cells could be induced to proliferate *in vitro* in the presence of proliferative and regulatory factors.

***Claims 9 and 10***

The Examiner relied on the combination of Boss, Anchan, Lin, Morshead and Gage to reject claims 9 and 10. The Examiner stated that:

The modification of the method of Boss, Anchan, Lin, Ferrari and Morshead by using human cells derived from humans having a neurological disorder as suggested by Gage in order to obtain a method for regulating the in vitro proliferation of a multipotent neural stem cell was within the ordinary skill in the art at the time the claimed invention was made. (p. 6, bottom ¶ of office action)

However, the Gage patent does not teach the use of human neural cells. Rather, Gage used fibroblasts (non-neural) cells. Gage does not suggest that it was possible to proliferate human multipotent neural stem cells in culture that are capable of producing progeny that can differentiate into neurons, astrocytes and oligodendrocytes. In fact, Gage indicated that there was a "paucity of replicating non-transformed cell culture systems" which could be used for neural transplantation purposes (see col. 14, lines 61-66). Thus, rather than attempting to culture neural cells, Gage used fibroblasts. Accordingly, one of ordinary skill in the art would not have modified the method of Boss by using the cells of Gage because the two references concern two different types of cells. Moreover, even if one were to have modified the prior art in the

Serial No.: 08/338,730  
Filed: 14 November 1994


manner suggested by the Examiner, it would not have resulted in the claimed invention.

**CONCLUSION**

For the foregoing reasons, it is believed that the Examiner's rejections should be withdrawn.

Respectfully submitted,

FLEHR, HOHBACH, TEST,  
ALBRITTON & HERBERT

  
Jan P. Brunelle  
Reg. No. 35,081

Four Embarcadero Center  
Suite 3400  
San Francisco, CA 94111-4187  
Telephone: (415) 781-1989

Dated: 5 July 1996